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Etanercept and Infliximab, Two Tumor Necrosis Factor-α (TNF-α) Inhibitors With Different Action Profiles: An *In vitro* and *In Vivo* Study in the Context of Reviewed Therapeutic Applications

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Abstract: Tumor necrosis factor-α (TNF-α) is a cytotoxic cytokine *in vitro*. It mediates septic shock, apoptosis, and inflammation *in vivo*. The *in vitro* work was used for testing the effect of Etanercept (ETA) and Infliximab (INF) against cell cytotoxicity caused by TNF-α. At the same time, the *in vivo* work was used to test the effect of the two tested agents on TNF-α serum levels in rats induced by lipopolysaccharides (LPS). Upon *in vitro* testing of the protective effect of both agents on the WI38 cells, it was found that ETA was superior to INF for antagonizing the effect of TNF-α whether the tested agent and TNF-α were simultaneously added to the cell line or the antibody addition proceeded TNF-α addition. *In vivo* testing of the effect of both agents showed that they significantly antagonized the effect of TNF-α with superior effect in case of ETA whether each tested agent was administered at four doses separated by one-week interval before LPS injection or it was administered once 24 hours after LPS administration. This study gave evidence for a differential antagonizing effect of ETA and INF against TNF-α with superior action in the case of ETA when they were assessed both *in vitro* and *in vivo*.

Keywords: TNF-α; cytotoxicity; Etanercept; Infliximab; WI38 cell line; laboratory animals.

1. Introduction

Tumor necrosis factor (TNF) is a cytokine produced by macrophages that mediates inflammation and apoptosis [1,2]. It is involved in the pathogenesis of several inflammatory diseases, such as Crohn's disease (CD) [3], rheumatoid arthritis (RA) [4], systemic lupus erythematosus (SLE) [5], ulcerative colitis, multiple sclerosis (MS), and systemic sclerosis [6]. It is also involved in viral replication, septic shock, tumor genesis, inflammation, and autoimmunity [7] and contributes to pathological and physiological processes [8]. It has a role in normal physiology, chronic inflammation, acute inflammation, cancer-related inflammation, and autoimmune diseases [9]. Tumor necrosis factor-alpha (TNF-α) is the most widely studied cytokine of the TNF superfamily [10]. This factor is formed by many cell types: immune cells

such as macrophages and monocytes, neutrophils, natural killer cells (NK), T-lymphocytes, fibroblasts, and neuronal cells. As well as its principal role in inflammatory events, there is increasing confirmation for the participation of TNF-α in cytotoxicity. Injection of lipopolysaccharides into rats stimulates the secretion of TNF-α from macrophages [11,12]. This cytokine occurs in two biologically active forms; the first is a transmembrane protein (tmTNF) which can be degraded by the metalloproteinase TNF-α-converting enzyme (TACE) into its second form, a soluble TNF (sTNF) [13]. There are two TNF-binding transmembrane receptors, namely the TNF receptor 1 (TNFR1 or CD120a) and the TNF receptor 2 (TNFR2 or CD120b) [14]. TNFR1 is expressed universally on almost all cell types, whereas TNFR2 is restricted to immune cells and some tumor cells [15]. Monoclonal anti-TNF antibodies are evaluated for their ability to protect cells from the cytotoxic effects of human TNF-α. Antibodies that bind to the TNF receptor have been shown to prevent cytotoxicity. Anti-TNF-α antibodies are used to prevent the binding of TNF-α to its receptor and to promote its clearance from circulation via antigen-antibody complexes [16,17]. Anti-TNF-α antibodies have also been shown to neutralize the direct cell-to-cell killing mediated by the membranous form of TNF-α [18,19]. Two TNF-α inhibitors, ETA (a p75 TNF-α receptor/Fc fusion protein) and INF (a chimeric monoclonal antibody), have been allowed to treat rheumatoid arthritis. INF (trade name Remicade) is a chimeric monoclonal antibody consisting of a murine variable region and a human IgG1 constant region [20]. INF was initially recognized in mice as a mouse antibody [21,22]. The common mouse domains were exchanged with similar human antibody domains as humans have immune reactions to mouse proteins. Because they were a combination of mouse and human antibody amino acid sequences, they are called a "chimeric monoclonal antibody" [23]. ETA (trade name Enbrel) is a soluble fusion protein consisting of the extracellular domain of fully human TNFR2 and Fc domain of human immunoglobulin G1 (IgG1). [24-26]. The Fc domain was commonly used to extend the serum half-life of the engineered protein. ETA binds specifically with soluble circulating TNF-α and prevents it from binding to its receptor, thus decreasing the biological activity of TNF-α. As a result, it has the potential to treat various autoimmune diseases or disorders associated with increased TNF-α and excess inflammation [27]. TNF-a inhibitors are effective treatment important options for patients suffering from several immune-mediated inflammatory diseases such as ankylosing spondylitis (AS), inflammatory bowel disease (rheumatoid arthritis (RA), Crohn's disease, psoriasis, and ulcerative colitis) [28-30]. These biological therapeutics are either receptor fusion proteins (ETA) that suppress the physiologic response to TNF-α or monoclonal antibodies (INF, Adalimumab, Certolizumab pegol, Golimumab). Although ETA Adalimumab, Golimumab, and INF are all inhibitors of TNF-α, they influence body composition and weight differently [31]. The first TNF-α blocker used clinically to treat RA was INF [32].

Our study aimed to evaluate the effectiveness of the two TNF-α inhibitors, ETA and INF, for their capacity to protect cells from the cytotoxic effect of this pleiotropic inflammatory agent and evaluate their effectiveness in the reduction of TNF-α serum levels in rats induced by lipopolysaccharide. The results reported here showed that ETA was superior to INF at 10 µg/ml while the latter was superior at 5 µg/ml concentration when testing their protective effect against the cytotoxicity caused by TNF-α on the WI38 cell line. *In vivo* testing showed that both ETA and INF significantly reduced TNF-α serum levels in laboratory animals induced with LPS after or before their administration with superior action in case of ETA. As a result,

both ETA and INF showed prophylactic and therapeutic antagonizing effects against TNF-α. The obtained results were interpreted in the context of reviewed therapeutic applications.

2. Materials and Methods

2.1. Chemicals and reagents.

The chemicals used in the present study included: Human Recombinant TNF-α (National Institute for Biological Standards and Control (NIBSC, United Kingdom)), 3-(4,5-Di-methylthioazole-2-yl) 2,5-Diphenyl tetrazolium bromide (MTT dye) (HIMEDIA, Mumbai, India), Actinomycin D (Sigma Aldrich, Saint Louis, USA), Trypsin 0.25% in phosphate buffer saline (PBS), Bovine serum albumin (BSA) (Serva Electrophoresis GmbH, Heidelberg, Germany), Sodium dodecyl sulfate (SDS) (Sigma Aldrich, Saint Louis, USA), Dimethyl sulfoxide (DMSO) (Sigma Aldrich, Saint Louis, USA), Fetal bovine serum (FBS) and Roswell Park Memorial Institute medium (RPMI) (GIBCO, New York, USA), Dulbecco's Modified Eagle Medium (DMEM) (Biowest, Le Vieux-Bourg, France), Hydrochloric acid (HCl) (Sigma Aldrich, Saint Louis, USA)

2.2. Antibodies.

Remicade (INF), a chimeric (mouse-human IgG) monoclonal anti-TNFα (GlaxoSmithKline, Brentford, United Kingdom), Enbrel (ETA), a genetically engineered fusion protein of recombinant human TNF receptor fused with FC domain of human IgG (GlaxoSmithKline, Brentford, United Kingdom)

2.3. Cell line and its maintenance.

The cell lines used in this study were the HeLa cell line (Cervic adenocarcinoma epithelial cells) and the WI38 cell line (Human normal lung Fibroblast Cells) were supplied by the tissue culture unit at the Egyptian Holding Company for Vaccines and Sera (VACSERA) (Giza, Egypt). The original source of cell lines was the American Type Tissue Culture Collection (ATCC) (Gaithersburg, Maryland). The cell lines were supplied in 75 cm^2 cell culture flasks with maintenance media RPMI enhanced with 10% FBS for the Hela cell line and DMEM enhanced with 10% FBS for the WI38 cell line.

2.4. In vitro determination of ETA and INF cytoprotective effect against TNF-α on the WI38 cell line.

2.4.1. Determination of sensitivity of HeLa and WI38 cell lines to tumor necrosis factor.

Cells were harvested into RPMI medium (for Hela cell line) or DMEM (for WI38 cell line) containing 0.1 mg/ml streptomycin, 100 U/ml penicillin, and 10% fetal bovine serum. An aliquot (50 µl) containing the indicated number of cells was placed into each well of a 96-well microtiter plate. The tested cell numbers were [(1-8)x10^5] cells/ml. Incubation was done at 37°C for 4 h. Following incubation, 50 µl aliquot per each well of TNF-α solution dissolved RPMI medium containing 2 µg/ml actinomycin D was added. TNF-α was used in concentrations: 10, 1, 0.1 and 0.01 ng/ml. The plates were incubated for a further 24 h at 37°C, the cells overlaid medium was removed and replaced with 50 µl of 5 mg/ml MTT solution per well. The plates were re-incubated at 37°C for 4 h, followed by adding to each well 50 µl

DEMSO to dissolve the dye or its transformed derivative. The absorbance of the resulting color was read at 570 nm [33].

2.4.2. Determination of the cytoprotective effect of synchronous addition of ETA or INF with TNF-α to the tested cell line.

The cells of cell line type proved to be sensitive to TNF-α (WI38 cell line) and were harvested into DMEM medium containing 100 IU/ml penicillin, 0.1 mg/ml streptomycin, and 10% fetal bovine serum. Aliquots (50 µl each) containing the indicated number of cells were distributed into the wells of a 96-well microtiter plate. The number of cells used was 2×10^5 cells/ml. Incubation was done at 37°C for 4 h, followed by the addition of aliquots (50 µl each) of TNF-α/MCA mixture in DMEM medium containing 2 µg/ml actinomycin D. The antibodies used were Remicade (100 mg/10 ml) vial and Enbrel (50 mg/1ml) prefilled syringe (PFS). TNF-α was used at a 5 ng/ml concentration (this concentration produced about 38.9% cell death). To prepare antibodies and TNF-α for addition to the cells in the monolayer, the antibodies were serially diluted into a medium containing 2 µg/ml actinomycin D to give 20, 10, 5, and 2.5 µg/ml antibody concentrations. An equal volume of 5 ng/ml TNF-α in the same media was added to the diluted antibodies. After incubation for 2 h, 50 µl of TNF-α/MCA mixture was added to the cell. The plates were incubated for 24 h at 37°C, and then the cytotoxicity was determined by MTT assay.

2.4.3. Determination of the protective effect of pre-incubation with ETA or INF against the cytotoxic effect of TNF-α on the tested cell line.

WI38 cell line was harvested and grown in the same culture medium as described before. Aliquots (50 µl each) of the various concentrations (20, 10, 5, 2.5 µg/ml) of each tested antibody (INF or ETA) were added to the grown WI38 cell line after discarding the overlaid medium. The plates were incubated for 4 h at 37°C, followed by adding 50 µl aliquots of TNF-α (5 ng/ml) to the microtitre plate wells. The plates were pre-incubated under the same conditions for 24 h, and then the cytotoxicity was determined by MTT assay.

2.5. In vivo determination of ETA and INF effect on serum level of TNF-α in laboratory animals induced with LPS.

2.5.1. Animals.

Adult albino Wister rats weighing 150 - 200 gm were used throughout the experiments. Animals were accommodated under standard environmental conditions (55±5% humidity, 23 ± 1ºC, a 12 h dark cycle, and a 12 h light) and maintained with free access to water and a standard laboratory diet ad libitum. The National Research Centre Animal Care and Use Committee accepted the experimental protocols and animal care. They were following the International ethical guidelines for the Study of Pain Committee.

2.5.2. Procedures.

2.5.2.1. LPS and antibody administration.

Lipopolysaccharides (Escherichia coli, serotype O111: B4) were purchased from Sigma-Aldrich, Germany. Two different approaches were conducted. In each approach, a

group of 10 rats (150-200 gm.) were used, the control group received normal saline, and the serum level of TNF-α was determined from the blood sample withdrawn at the beginning and at the end of the experiment (24 h after LPS injection in the first approach and 15 days after the tested antibody injection in the second approach). In the first approach, the animals were injected at 8 mg/kg/week ETA and 7 mg/kg/week INF. The test drug was administered on the 1st, 7th, 14th, and 21st days then LPS was injected at 4 mg/kg on the 28th day of the experiment. The animals were kept under standardized conditions of feeding and accommodation. Blood samples were withdrawn 24 h after LPS administration. In the second approach, the animals were kept under standardized conditions of feeding and accommodation for 7 days then LPS was injected at 4 mg/kg. Then, after 24 h, both ETA and INF were administered at 8 and 7 mg/kg, respectively. Blood samples were withdrawn on the 15th day after the antibody administration.

2.5.2.2. Collection of blood samples and serum preparation.

At the end of the experimental period, blood samples were collected using heparinized capillary tubes. Animals fasted for 12 h before blood collection. Blood samples were allowed to clot at room temperature, and then the serum was separated by centrifugation at 3000 rpm for 15 minutes. Serum aliquots were stored at -80ºC in a deep freezer until assayed for TNF-α level.

2.6. Statistical analyses.

Statistical analyses of the data were achieved using one-way ANOVA followed by Tukey's multiple comparisons test.

2.7. List of abbreviations.

MCAs: monoclonal antibodies; ETA: Etanercept; INF: Infliximab; TNF-α: Tumor Necrosis Factor-α; LPS: lipopolysaccharides; RA: rheumatoid arthritis; CD: Crohn's disease; SLE: systemic lupus erythematosus; MS: multiple sclerosis; CB: chronic bronchitis; COPD: chronic obstructive pulmonary disease; ALI: Acute lung injury; ARDS: acute respiratory distress syndrome; tmTNF: transmembrane protein; TACE: TNF-α-converting enzyme; sTNF: soluble TNF; TNFR1: TNF receptor 1; TNFR2: TNF receptor 2; WI38 cell line: Wister Institute 38; Hela cell line: Henrietta Lacks MTT: 3-2,5 diphenyltetrazolium-bromide; PBS: phosphate buffer saline; IgG1: immunoglobulin G1; PBS: phosphate buffer saline; BSA: Bovine serum albumin; SDS: sodium dodecyl sulfate; DMSO: dimethyl sulfoxide; FBS: fetal bovine serum; RPMI: Roswell Park Memorial Institute medium; DMEM: Dulbecco's Modified Eagle Medium.

3. Results and Discussion

3.1. Results.

3.1.1. The cytoprotective effect of ETA and INF as determined by the *in vitro* study on cell lines exposed to TNF-α.

In this study, we first examined the sensitivity of two cell lines, HeLa and WI38, to TNF-α. The results showed that the HeLa cell line was not sensitive to the action of the TNF-

α (data not shown), while the WI38 cell line was sensitive to that action (Figure 1). The cytotoxic effect of TNF-α depended on the number of cells exposed to TNF-α and the concentration applied to this agent. The greatest effect was obtained at 2×105 cells/ml cell count and 10 ng/ml TNF-α concentration. However, the cytotoxicity at 1 ng/ml TNF-α was still high. Accordingly, the WI38 cell line at a cell count of 2×10^5 cells/ml was used to study the cytoprotective effect of the two tested monoclonal antibodies on the action of TNF-α at 5 ng/ml concentration.

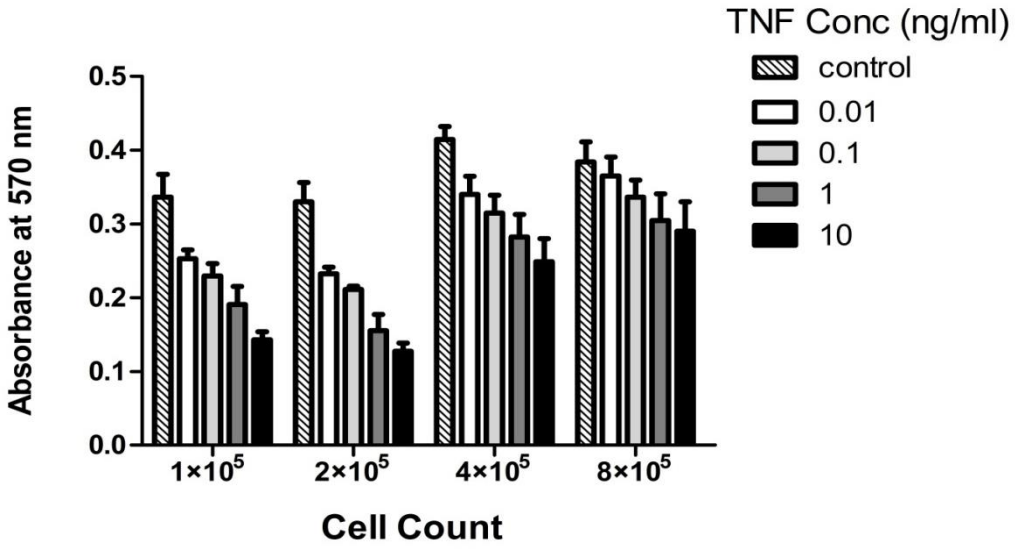

Figure 1. Effect of TNF-α on WI38 cell line viability after 24 h exposure time.

To measure the ability of ETA and INF to protect against TNF-α action, each tested antibody was titrated against the action of TNF-α at 5 ng/ml on the WI38 cell line at a cell count of 2×10^5 cells/ml. This was carried out using two addition regimens; the first regimen included the simultaneous addition of each tested monoclonal antibody with TNF-α to the WI38 cell line. While in the second regimen WI38 cell line was first exposed to each tested antibody for four hours, followed by the addition of TNF-α. As a validation step, exposure of the WI38 cell line to TNF-α at 5 ng/ml produced cell cytotoxicity of 38.9%, while exposure of the tested cell line to each of ETA and INF at 10 µg/ml each caused no observable cytotoxicity. Upon simultaneous exposure of the tested cell line to ETA and TNF-α or INF and TNF-α, the cytotoxicity of TNF-α decreased (Figure 2A). The reduction in cytotoxicity caused by simultaneous use of ETA and TNF-α was higher than that caused by simultaneous use of INF and TNF-α at 10 µg/ml of each antibody. Reducing the concentration of each tested antibody to 5 µg/ml still showed cytoprotective activity against the action of TNF-α on the WI38 cell line. Still, this activity was lower than that observed at each tested antibody's 10 µg/ml concentration. Surprisingly, the cytoprotective effect caused by INF at 5 µg/ml was higher than that exerted by ETA, a result opposite to that seen for both tested antibodies at 10 µg/ml (Figure 2A & B). However, further reduction of each tested antibody concentration to 2.5 µg/ml showed nearly no cytoprotective action when each concomitantly added with TNF-α to WI 38 cell line (Figure 2C).

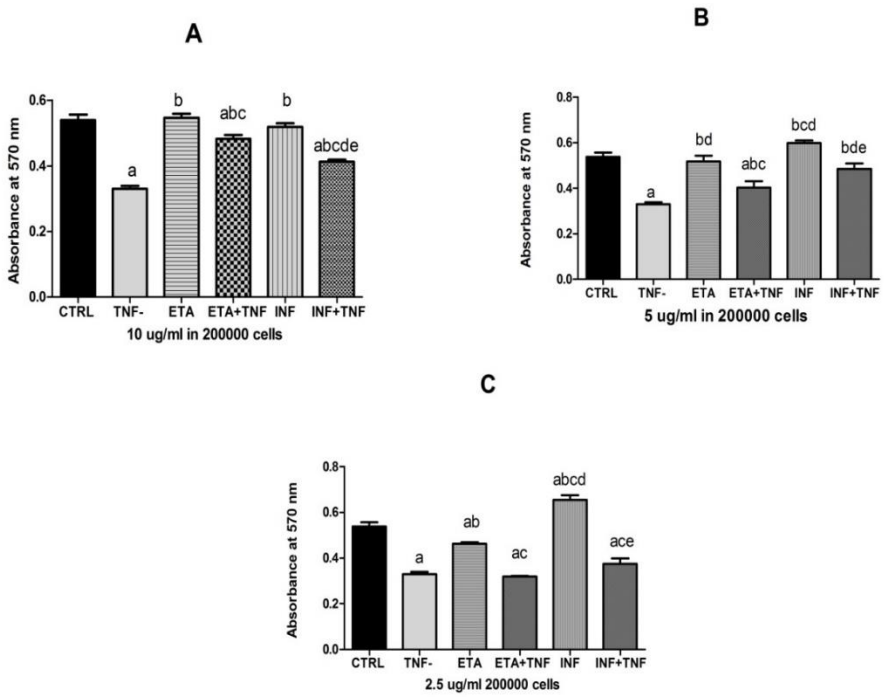

Figure 2. The cytoprotective effect of Etanercept and Infliximab when each was simultaneously added with TNF-α to WI38 cell line at 10 µg/ml (A), 5 µg/ml (B), and 2.5 µg/ml (C). TNF-α was used at 5 ng/ml, and the WI38 cell line was used at 2×10^5 cells/ml. Data are presented as means ± SEM. (a):significantly different from control; (b): significantly different from TNF-α; (c): significantly different from ETA; (d): significantly different from ETA+ TNF-α; (e): significantly different from INF, each at $p < 0.05$. CTRL: control; TNF-α: tumor necrosis factor-α; INF: Infliximab; ETA: Etanercept

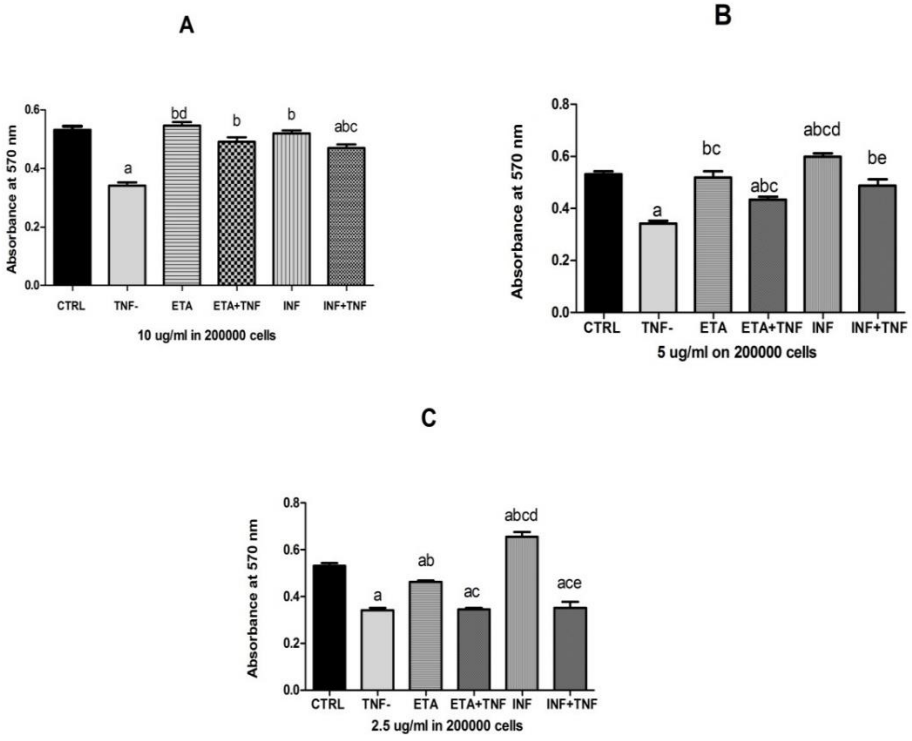

Figure 3. The cytoprotective effect of Etanercept and Infliximab when each was added to WI38 cell line at 10 µg/ml (A), 5 µg/ml (B), and 2.5 µg/ml (C) followed by the addition of TNF-α. TNF-α was used at 5 ng/ml and the WI38 cell line was used at 2×10^5 cells/ml. Data are presented as means ± SEM. (a): significantly different from control; (b): significantly different from TNF-α; (c): significantly different from ETA; (d): significantly different from INF; (e): significantly different from INF, each at $p < 0.05$. CTRL: control; TNF-α: tumor necrosis factor - α; INF: Infliximab; ETA: Etanercept.

In the second regimen, the WI38 cell count and 5 ng/ml TNF-α produced about 35.6% cell cytotoxicity. Upon exposure of the tested cell line to ETA or INF for four hours, followed by the addition of TNF-α, cytotoxicity of TNF-α decreased (Figure 3A). Again, the reduction in cytotoxicity caused by exposure of the tested cell line to ETA was higher than that caused by cell line exposure to INF at 10 µg/ml of each antibody. Reducing the concentration of each tested antibody to 5 µg/ml still showed cytoprotective activity against the action of TNF-α on the WI38 cell line. Still, this activity was lower than that observed at 10 µg/ml concentration for each tested antibody. Surprisingly, the cytoprotective effect caused by INF at 5 µg/ml was higher than that exerted by ETA (Figure 3A & B). However, further reduction of each tested antibody concentration to 2.5 µg/ml showed nearly no cytoprotective action (Figure 3C).

3.1.2. Effect of ETA and INF on serum level of TNF-α as determined by the *in vivo* study in laboratory animals induced with LPS.

This was carried out using two different approaches; the first approach included administering each tested antibody (ETA and INF) to the laboratory animals at four doses separated by one week before LPS injection, as shown in the method section. In the second approach, LPS was administered first; 24 h later, each antibody was injected once. The baseline Serum level of TNF-α of each tested rat was first determined pretreatment with each tested antibody, and pre-administration of LPS was within the same range as a control group. Pretreatment of the used animals with each tested antibody caused a significant reduction in the serum level of TNF-α in LPS-induced rats (Figure 4). However, the reduction in serum level of TNF-α caused by ETA administration at 8 mg/ml/week for four weeks was significantly higher than that caused by INF at 7 mg/kg/week at the same dose schedule a result comparable to that obtained *in vitro* experiment. Interestingly, ETA decreased the serum level of TNF-α of the tested animals induced by LPS to its normal level in the absence of LPS induction.

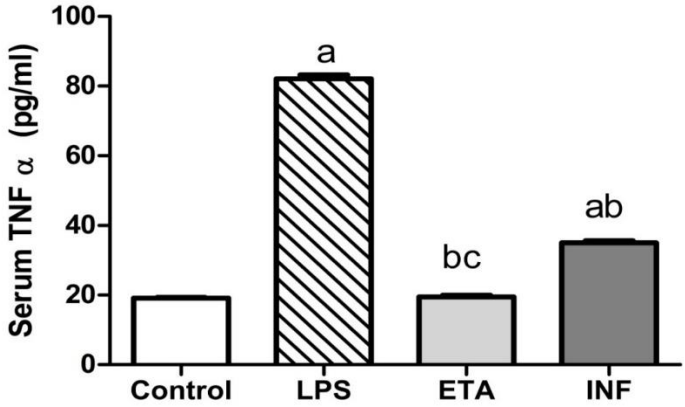

Figure 4. Effect of administration of Etanercept (8 mg/kg/week, i.p.) and Infliximab (7 mg/kg/week, i.p.) when each was given at a one-week interval for four doses on TNF-α serum level of rats injected with LPS (4 mg/kg, i.p) after antibody administration. Data are presented as means ± SEM. (a): significantly different from control; (b): significantly different from LPS group; (c): significantly different from ETA, each at $p < 0.05$.

Pre-administration of LPS and 24 h later injection of each tested antibody once caused a significant reduction in serum level of TNF-α of LPS-induced rats (Figure 5). Again, the reduction in serum level of TNF-α caused by ETA administration at 8 mg/ml as a single dose was significantly higher than that caused by INF at 7 mg/kg as a single dose. Interestingly, ETA decreased the serum level of TNF-α of the tested animals induced by LPS to its normal level in the absence of LPS induction.

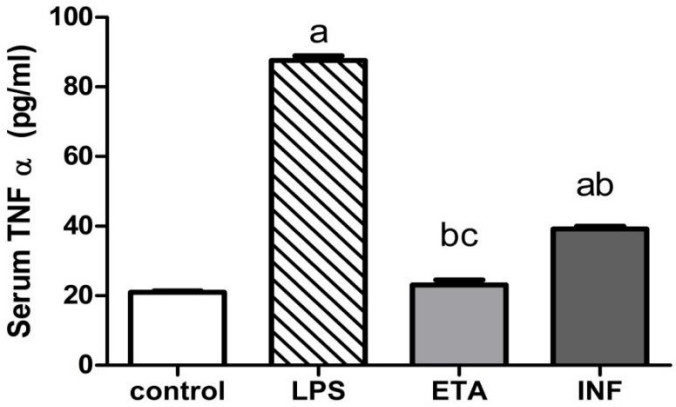

Figure 5. Effect of administration of Etanercept (8 mg/kg, i.p) and Infliximab (7 mg/kg, i.p.) on TNF-α serum level of rats when each was given once 24 hours after LPS injection (4 mg/kg, i.p). Data are presented as means ± SEM. (a): significantly different from control; (b): significantly different from LPS group; (c): significantly different from INF, each at $p < 0.05$.

3.2. Discussion.

Expression of Tumor necrosis factor-α (TNF-α) increased in a variety of cancers, and this correlates with many tumor features such as higher malignancy grade, augmented cell proliferation, increased occurrence of metastasis, and poor prognosis for the affected patient, as in the case of breast cancer. In clinic settings, TNF-α blocking agents are used to treat inflammatory and autoimmune diseases [34-36]. Patients suffering from multiple autoimmune diseases showed elevated expression levels of TNF-α [37,38]. Antigen-binding fragments (Fab) and scavenging antibodies against TNF-α proved to successfully suppress mediated inflammation caused by TNF-α- in inflammatory autoimmune diseases such as psoriasis, Crohn's disease, and rheumatoid arthritis[39,40]. For the treatment of RA, INF was the first approved anti-TNF-α monoclonal antibody [41-43]. This agent is given intravenously. On the contrary, ETA, the recombinant version of the soluble p75 TNF-α receptor, and Adalimumab, the anti-TNF-α monoclonal human antibody, are administered subcutaneously. In the present study, we studied the effect of two TNF-α inhibitors approved for human use to protect the WI38 cell line against TNF-α cytotoxicity in vitro and their efficiency in lowering or omitting TNF-α levels in lipopolysaccharide-induced mice *in vivo*. The results obtained were interpreted in the context of the therapeutic application of these two agents and other TNF-α biologic inhibitors.

For testing the cytoprotective effect of ETA and INF for cell lines exposed to TNF-α, we first examined the sensitivity of two cell lines, HeLa and WI38, to TNF-α. While the HeLa cell line showed no sensitivity to the action of the TNF-α at the tested concentrations, the WI38 cell line did. The cytotoxic effect produced was both cell line count- and TNF-α concentration-dependent. The greatest effect was obtained at 2×10^5 cells/ml cell count and 10 ng/ml TNF-α concentration. It was reported that various human tumor cell lines of different histological origins don't behave alike regarding their sensitivity and resistance to tumor necrosis factor-α (TNF-α) and Adriamycin (ADR). Watanabe *et al*. tested six ovarian lines, one of a lung, renal, and B-cell line, for their resistance mechanisms to TNF-α and ADR [44]. They found that no overall correlation exists for the cytotoxicity of these agents against the tested cell lines. The combination of ADR and TNF-α showed increased cytotoxicity against the tested cell lines. The authors proposed a resistance mechanism in tumor cells was the endogenous production of TNF-α mRNA and protein. A positive correlation between the constitutive production of TNF-α mRNA and protein and the resistance to TNF-α was detected. There was an up-

regulation of TNF-α mRNA in the presence of TNF-α for the three TNF-α sensitive tumor lines. The authors concluded that TNF-α is highly cytotoxic for some tumor lines but of little or no effect on others [44]. However, the mechanism of resistance to the cytotoxic effect of TNF-α has been related to many findings such as overexpression of the HER2 oncogene [45], the production of free radical scavengers like MnSOD [46], the induction of expression of TNF mRNA itself [47] and differential degradation of TNF-α in sensitive versus resistant cells [48]. Gapuzan *et al*. reported that NF-kB has a key role in preventing apoptosis induced by tumor necrosis factor-α (TNF-α) [49]. Upon inhibiting NF-kB activity by overexpression of its repressor, the intratumoral administration of TNF-α produced regression of tumors in human fibrosarcoma cells [50]. After evaluating non-small cell lung cancer (NSCLC) cell lines' resistance to TNF-α, it was suggested that Ni sensitizes NSCLC cell lines to induce cell death caused by TNF-α by inhibiting NF-kB protein expression and activation [51]. Some authors suggested that differences in susceptibility of MCF-7 breast cancer cell variants to TNF-α-induced apoptosis could be attributed to the differences in ceramide generation, TNFR expression, protease activation, and differential expression of the Bcl-2 family of proteins [52]. Pagliacci *et al*. showed that the cytotoxic versus the cytostatic effects of TNF-α rely on serum conditions and media used to culture the MCF-7 cells [53]. However, in our study, for measuring the ability of ETA and INF to protect against TNF-α action, WI38 cell line at cell count 2×10^5 cells/ml while TNF-α was applied at 5 ng/ml concentration. Upon simultaneous exposure of the tested cell line to ETA and TNF-α or INF and TNF-α, the cytotoxicity of TNF-α decreased (Figure 2A) compared to control (no antibodies were added). The reduction in cytotoxicity caused by ETA was higher than in the case of INF. Reducing the concentration of each tested antibody to 5 µg/ml still showed cytoprotective activity against the action of TNF-α on the WI38 cell line. Still, this activity was lower than that observed at the 10 µg/ml concentration of each tested antibody. The use of ETA at 10 µg/ml reduced the cytotoxicity caused by TNF-α by 58.4% in the first regimen and 79.2% in the second regimen. However, 5 µg/ml ETA caused about a 35% reduction in TNF-cytotoxicity in the first regimen and 60% in the second regimen. The cytoprotective effect caused by INF at 5 µg/ml was higher than that exerted by ETA. In the case of INF and surprisingly, no significant effect in TNF-cytotoxicity was observed upon its use at 10 µg/ml in both regimens, while the similar treatment at 5 µg/ml caused about 73.8% reduction in TNF-cytotoxicity in the first regimen and 77.5% reduction in TNF-α cytotoxicity in the second regimen. Therefore, the reduction in cytotoxicity caused by the use of ETA was higher than that produced in the case of INF at 10 µg/ml while the opposite action was obtained at 5 µg/ml concentration. From the obtained results, it can be inferred that: (i) the protective effect of each tested TNF-α inhibitor is concentration-dependent, (ii) the shown opposite action profile of the two inhibitors at 5 µg/ml concentration as compared to that at 10 µg/ml while TNF-α concentration was constant could be attributed to the structural differences between ETA and INF and consequently the binding pattern to TNF-α and the avidity of this binding as well as the binding kinetics are different. It was reported that ETA binds only to the trimer form of soluble TNF-α, whereas INF binds to monomer and trimer forms. ETA forms relatively unstable complexes, while INF forms stable complexes with soluble TNF-α. ETA was able to form a 1:1 complex with the TNF-α trimer. In contrast, each INF molecule was supposed to bind to two TNF-α molecules[20].

The effect of ETA and INF on the serum level of TNF-α was determined by an *in vivo* study in laboratory animals induced with LPS either before or after treatment with the tested TNF-α inhibitor. For these experiments, TNF-α was measured by the ELISA technique.

Wallace and Stacey used the ELISA technique for measuring TNF-α for its cytotoxic effect on the human rhabdomyosarcoma cell line, KYM-1D4 [54]. ELISA was accepted as the conventional method for TNF-α quantitation after 1987 [55]. In clinical investigations and research, TNF-α measurement was done by the ELISA method [56]. ELISA, RIA, and bioassays were examined for measuring TNF-α. The bioassays are sensitive for measuring the bioactive forms of TNF-α, but they were found to be of low reproducibility and specificity and high cost [56]. Therefore, the authors stated that they are not suitable for routine measuring of TNF-α. The basic drawback of RIA methods is radioisotopes [56]. The main non-isotopic immunoassays for measuring TNF-α are ELISA's. These methods were reported to have good reproducibility and high sensitivity [55]. As in our study, Fries *et al*. determined serum concentrations of TNF-α by ELISA [57].

In vivo study experiments showed that either pretreatment of the used animals with each tested antibody before TNF-α injection or pre-administration of LPS followed by injection of each tested antibody caused a significant reduction in serum level of TNF-α of LPS induced rats, and the action of ETA was significantly higher than that caused by INF. Also, in both cases, ETA decreased the serum level of TNF-α of the tested animals induced by LPS to its normal level in the absence of LPS induction. Regarding the therapeutic effects of both ETA and INF, it was reported that the two TNF-α antagonists, ETA and INF, have been shown to be equally effective against rheumatoid arthritis, while Infliximab was the only effective one in Crohn's disease (another TNF-α mediated disease) [58]. The authors stated that in patients with Crohn's disease, INF, but not Etanercept, can bind to the transmembrane TNF-α on T cells of lamina propria. and only INF can induce apoptosis of activated lymphocytes [58]. Specific anti-TNF-α therapies have substantially improved the clinical course and outcome in the case of RA of the disease [59,60]. The five approved TNFα inhibitors include the anti-TNF-α antibodies INF, Adalimumab, and Golimumab, the TNFR2 Fc fusion protein Etanercept (ETA), and the PEGylated Fab fragment Certolizumab. They block TNF-α biological activity through competitive inhibition of TNF-α binding to its cognate cellular receptors. In RA, the unequivocal therapeutic effects of ETA and anti-TNF-α antibodies led to two conclusions: (i) TNF-α has an essential contribution to the immunopathogenesis of RA as anti- TNF-α therapies mediate their action by inhibiting TNF-α activity at the site of disease, (ii) increased risk of certain infections occurred among patients on anti-TNF-α therapies, a detrimental effect of TNF-α inhibition. Dixon *et al*. [61] suggested that the anti-TNF antibodies and ETA have differential biological activity. Although all mediate similar therapeutic action in RA, the anti-TNF-α antibodies but not ETA are effective in the case of Crohn's colitis [62]. Also, using anti-TNF-α antibodies but not ETA constitutes a greater risk of active tuberculosis [63]. Additionally, authors have previously shown that anti-TNF-α antibodies enhance the increased emergence of functionally active regulatory T cells. This effect has not been seen in the case of ETA [64,65]. Harris and Keane reported that two types of TNF-α antagonists are used for treating RA and spondyloarthritides; these include monoclonal antibodies such as Adalimumab, INF, Golimumab, or pegylated-IgG-Fc fragments such as Certolizumab and the soluble TNF-α receptor 2 ETA. Regarding the possible risk of serious infection (especially tuberculosis reactivation), ETA is considered the safest anti-TNF-α inhibitor. Several studies have shown that INF and Adalimumab monoclonal antibody therapy but not the recombinant soluble TNF-α receptor ETA increase the risk of reactivation of latent tuberculosis [66]. The safety profile of both types of TNF-α inhibitors may differ in part as a result of their TNF-α binding property. ETA interacts with soluble TNF-α and only weakly and reversibly with its

transmembrane form, while INF forms stable complexes with soluble TNF-α, and TNF-α expressed at cell membranes [20,67]. Markatseli *et al*. showed that all three biological agents (Adalimumab, INF, and ETA) are effective in RA patients and their efficacy [68]. In a study conducted by Desai *et al*., it was found the most frequently reported adverse event for Adalimumab, INF, and ETA was an infection, with the highest observed rate in the INF group (70.27%). Also, the INF group showed significantly more frequent systemic allergic reactions (39.19%) than Adalimumab (2.67%). In contrast, ETA group, no allergic reactions were observed. In RA patients, the risk of treatment discontinuation as a result of adverse events was greater for INF than for Adalimumab, and the least was for ETA [69]. Flouri *et al*. showed that a higher rate of antibodies against INF compared with Adalimumab and ETA could explain the higher incidence of allergic reactions. The higher occurrence of infections may be due to structural and pharmacological differences among the three drugs. Although survival for all three treatments was satisfactory, it was significantly lower in the case of INF compared with Adalimumab and ETA [70]. Leon *et al*. also reported the same result [71]. The possible explanation is the high incidence of anti-drug antibodies (ADAs) demonstrated in the case of INF compared to the other two drugs, causing a higher rate of allergic reactions and inefficacy [72]. ADAs were reported to neutralize TNF-α inhibitors' activity and can provoke serious adverse events, such as allergic reactions and vasculitis [73]. Though IgG1 and IgG4, which are not involved in allergic reactions, are the most common types of ADAs, other ADAs types (IgA, IgM, and IgE) have also been detected [73]. Researchers detected IgE INF ADAs in patients who experienced a hypersensitivity reaction to INF [74]. Lower survival of INF compared to Adalimumab was recorded as a consequence of its side effects, especially infections, and ETA recorded a lower percentage of discontinuation [75]. In this respect, other supporting studies were conducted [68,76]. It was reported that TNF-α inhibitors demonstrate a satisfactory safety profile. Still, RA patients treated with INF are more likely to discontinue treatment earlier than the other alternatives, Adalimumab and ETA. Infections and allergic reactions appear more often with INF. However, the three drugs did not differ in serious infection occurrence [77]. As an adjunct feature for ETA, studies showed that except for both Certolizumab and ETA, all anti-TNF-α agents could induce antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent (CDC) [78,79]. Two studies approved by the same laboratory found decreased and increased Th1 cells in the blood of ankylosing spondylitis (AS) patients treated with ETA or INF, respectively [80]. As suggested by the authors, this differential effect may be associated with the different efficacy of these two compounds in treating Crohn's disease. Additionally, this may be linked to the distinct rates of tuberculosis infections following treatments by these two agents. It was reported that Tumor necrosis factor-alpha (TNFα) might paradoxically induce either apoptosis or cell survival. It exerts its activity by binding TNF-receptor (TNFR) 1 or 2. TNFR1 essentially transmits apoptotic signals. Depending on the type of TNFR and the subsequent recruitment of the related intracellular adapter proteins, TNF-α can induce controversial signals such as pro-inflammatory/anti-inflammatory or apoptotic/anti-apoptotic. TNFR1 involvement can induce or inhibit apoptosis [81,82]. Apoptosis inhibition is mediated through activation of NF-κB [83]. Unlike TNFR1, TNFR2 lacks a death domain; consequently, the transduction of TNF-α signaling via TNFR2 produces an anti-apoptotic effect and inflammation through NF-κB activation [84,85]. Anti-TNF-a therapy resulted in a rapid improvement in clinical outcomes. The good/moderate response rate was 82% for INF, 89.6% for Adalimumab, and 95.6% for ETA. The withdrawal rate was 80% for patients on INF, 61.4% for patients on Adalimumab, and 47.6% for patients

on ETA. The most common reasons behind discontinuation were allergic reactions for Infliximab (rate of discontinuation of 25.7%) and inefficacy for Adalimumab and ETA (17.5% and 23.8%, respectively). The incidence of infections and systemic allergic reactions was more frequent in the INF group. The three drugs did not differ in serious infection occurrence. According to Kaplan–Meier survival analysis, a significantly faster withdrawal for INF patients was recorded than for Adalimumab and ETA [77].

4. Conclusions

This study showed that ETA and INF could alleviate TNF-α cytotoxicity on mammalian cells represented by the WI38 cell line when tested *in vitro*. Both antibodies produced their cytoprotective effect when applied to the cell line before exposure to - or with the concomitant use of TNF-α. *In vivo* experiments revealed that ETA and INF could significantly reduce the TNF-α serum level of laboratory animals induced by lipopolysaccharides. This effect was demonstrated when the animals were treated with antibodies before or after lipopolysaccharide administration. ETA exerted superior cytoprotective activity in the *in vitro* experiment and in the reduction of TNF-α serum level in the *in vivo* experiment compared to INF. ETA but not INF decreased the serum level of TNF-α of the tested animals induced by LPS to its normal level in the absence of LPS induction when it was applied either before or after lipopolysaccharide administration. The evidence supports the continued use of both ETA and INF as therapeutic agents for TNF-α prevailing health conditions.

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Conflicts of Interest

The authors state no conflict of interest.

References

1. Boumaza, A.; Mezouar, S.; Bardou, M.; Raoult, D.; Mège, J.-L.; Desnues, B. Tumor Necrosis Factor Inhibitors Exacerbate Whipple's Disease by Reprogramming Macrophage and Inducing Apoptosis. *Frontiers in immunology* **2021**, *12*, 667357, https://doi.org/10.3389/fimmu.2021.667357.
2. Yao, L.; Pan, L.; Qian, M.; Sun, W.; Gu, C.; Chen, L.; Tang, X.; Hu, Y.; Xu, L.; Wei, Y. Tumor necrosis factor-α variations in patients with major depressive disorder before and after antidepressant treatment. *Frontiers in Psychiatry* **2020**, *11*, 518837, https://doi.org/10.3389/fpsyt.2020.518837.
3. Schreiber, S. Certolizumab pegol for the treatment of Crohn's disease. *Therapeutic Advances in Gastroenterology* **2011**, *4*, 375-389, https://doi.org/10.1177%2F1756283X11413315.
4. Lu, R.-M.; Hwang, Y.-C.; Liu, I.-J.; Lee, C.-C.; Tsai, H.-Z.; Li, H.-J.; Wu, H.-C. Development of therapeutic antibodies for the treatment of diseases. *Journal of biomedical science* **2020**, *27*, 1-30, https://jbiomedsci.biomedcentral.com/articles/10.1186/s12929-019-0592-z.

5. Aringer, M.; Smolen, J.S.Efficacy and safety of TNF-blocker therapy in systemic lupus erythematosus. *Expert opinion on drug safety* **2008**, *7*, 411-419, https://doi.org/10.1517/14740338.7.4.411.
6. Feldmann, M. Pathogenesis of arthritis: recent research progress. *Nature immunology* **2001**, *2*, 771-773, https://doi.org/10.1038/ni0901-771.
7. Faustman, D.L.; Davis, M. TNF receptor 2 and disease: autoimmunity and regenerative medicine. *Frontiers in immunology* **2013**, *4*, 478, https://doi.org/10.3389/fimmu.2013.00478.
8. Lee, K.H.; Ahn, B.S.; Cha, D.; Jang, W.W.; Choi, E.; Park, S.; Park, J.H.; Oh, J.; Park, H.; Park, J.H. Understanding the immunopathogenesis of autoimmune diseases by animal studies using gene modulation: a comprehensive review. *Autoimmunity Reviews* **2020**, *19*, 102469, https://doi.org/10.1016/j.autrev.2020.102469.
9. Jang, D.-i.; Lee, A.-H.; Shin, H.-Y.; Song, H.-R.; Park, J.-H.; Kang, T.-B.; Lee, S.-R.; Yang, S.-H. The role of tumor necrosis factor alpha (TNF-α) in autoimmune disease and current TNF-α inhibitors in therapeutics. *International journal of molecular sciences* **2021**, *22*, 2719, https://doi.org/10.3390/ijms22052719.
10. Guo, F.; Yuan, Y. Tumor necrosis factor alpha-induced proteins in malignant tumors: progress and prospects. *OncoTargets and therapy* **2020**, *13*, 3303, https://doi.org/10.2147%2FOTT.S241344.
11. Mukhopadhyay, S.; Hoidal, J.R.; Mukherjee, T.K. Role of TNFα in pulmonary pathophysiology. *Respiratory research* **2006**, *7*, 1-9, https://respiratory-research.biomedcentral.com/articles/10.1186/1465-9921-7-125.
12. Ren, B.; Lu, J.; Li, M.; Zou, X.; Liu, Y.; Wang, C.; Wang, L. Anti-inflammatory effect of IL-1ra-loaded dextran/PLGA microspheres on Porphyromonas gingivalis lipopolysaccharide-stimulated macrophages *in vitro* and *in vivo* in a rat model of periodontitis. *Biomedicine & Pharmacotherapy* **2021**, *134*, 111171, https://doi.org/10.1016/j.biopha.2020.111171.
13. Steeland, S.; Libert, C.; Vandenbroucke, R.E. A new venue of TNF targeting. *International journal of molecular sciences* **2018**, *19*, 1442, https://doi.org/10.3390/ijms19051442.
14. Mitoma, H.; Horiuchi, T.; Tsukamoto, H.; Ueda, N. Molecular mechanisms of action of anti-TNF-α agents–Comparison among therapeutic TNF-α antagonists. *Cytokine* **2018**, *101*, 56-63, https://doi.org/10.1016/j.cyto.2016.08.014.
15. Siegmund, D.; Kums, J.; Ehrenschwender, M.; Wajant, H. Activation of TNFR2 sensitizes macrophages for TNFR1-mediated necroptosis. *Cell death & disease* **2016**, *7*, e2375-e2375, https://doi.org/10.1038%2Fcddis.2016.285.
16. Atiqi, S.; Hooijberg, F.; Loeff, F.C.; Rispens, T.; Wolbink, G.J. Immunogenicity of TNF-inhibitors. *Frontiers in immunology* **2020**, *11*, 312, https://doi.org/10.3389%2Ffimmu.2020.00312.
17. Wang, Y.; Gao, Z.; Liu, Z.; Liu, G.; Qu, X.; Chen, J.; Ren, X.; Xu, Z.; Yang, H. A randomized, double-blind, single-dose, two-way, parallel phase I clinical study comparing the pharmacokinetics and safety of adalimumab injecta and Humira® in healthy Chinese male volunteers. *Expert Opinion on Biological Therapy* **2022**, *22*, 225-234, https://doi.org/10.1080/14712598.2021.1944097.
18. Li, W.; Liu, D.; Xu, J.; Zha, J.; Wang, C.; An, J.; Xie, Z.; Qiao, S. Astrocyte-Derived TNF-α Activated Platelets Promote Cerebral Ischemia/Reperfusion Injury by Regulating RIP1/RIP3/AKT Signaling Pathway. **2021**, https://link.springer.com/article/10.1007/s12035-022-02942-z.
19. Li, X.; Körner, H.; Liu, X. Susceptibility to Intracellular Infections: Contributions of TNF to Immune Defense. *Frontiers in Microbiology* **2020**, *11*, 1643, https://doi.org/10.3389%2Ffmicb.2020.01643.
20. Scallon, B. Cai A, Solowski N, Rosenberg A, Song XY, Shealy D, Wagner C. Binding and functional comparisons of two types of tumor necrosis factor antagonists. *J Pharmacol Exp Ther* **2002**, *301*, 418-426, https://doi.org/10.1124/jpet.301.2.418.
21. Gorelik, Y.; Freilich, S.; Gerassy-Vainberg, S.; Pressman, S.; Friss, C.; Blatt, A.; Focht, G.; Weisband, Y.L.; Greenfeld, S.; Kariv, R. Antibiotic use differentially affects the risk of anti-drug antibody formation during anti-TNFα therapy in inflammatory bowel disease patients: a report from the epi-IIRN. *Gut* **2022**, *71*, 287-295, https://doi.org/10.1136/gutjnl-2021-325185.
22. Pereira, I.R.; Vilar-Pereira, G.; Silva, A.A.; Moreira, O.C.; Britto, C.; Sarmento, E.D.M.; Lannes-Vieira, J. Tumor necrosis factor is a therapeutic target for immunological unbalance and cardiac abnormalities in chronic experimental Chagas' heart disease. *Mediators of inflammation* **2014**, *2014*, https://doi.org/10.1155%2F2014%2F798078.
23. Horiuchi, T. TNF-alpha: Structure, Function and Interaction With anti-TNF Agents. *Rheumatology (Oxford, № 49, c. 1215* https://doi.org/10.1093/rheumatology/keq031.
24. Moatti, A.; Cohen, J.L. The TNF-α/TNFR2 Pathway: Targeting a Brake to Release the Anti-tumor Immune Response. *Frontiers in Cell and Developmental Biology* **2021**, 2900, https://www.frontiersin.org/articles/10.3389/fcell.2021.725473/full.
25. Zeng, Y.; Huang, J.; Cui, C.; Yang, S.; Zhang, Z.-Y.; Ali, S.; Huang, Z.-J.; Yang, G.-P. QL0902, a proposed etanercept biosimilar: pharmacokinetic and immunogenicity profile to its reference product in healthy Chinese male subjects. *Expert Opinion on Biological Therapy* **2021**, *21*, 105-110, https://doi.org/10.1080/14712598.2020.1745182.
26. Zhang, H.; Shi, N.; Diao, Z.; Chen, Y.; Zhang, Y. Therapeutic potential of TNFα inhibitors in chronic inflammatory disorders: Past and future. *Genes & Diseases* **2021**, *8*, 38-47, https://doi.org/10.1016/j.gendis.2020.02.004.

27. van Schie, K.A.; Ooijevaar-de Heer, P.; Dijk, L.; Kruithof, S.; Wolbink, G.; Rispens, T. Therapeutic TNF inhibitors can differentially stabilize trimeric TNF by inhibiting monomer exchange. *Scientific reports* **2016**, *6*, 1-10, https://doi.org/10.1038/srep32747.
28. Barrera-Ochoa, C.A.; Berná-Rico, E.; Burgos-Blasco, P.; Saceda-Corralo, D.; González-Cantero, Á. Cardiovascular risk in immune-mediated inflammatory diseases. **2021**, https://www.researchgate.net/publication/356115196_Cardiovascular_risk_in_immune-mediated_inflammatory_diseases.
29. Cohen, B.L.; Sachar, D.B. Update on anti-tumor necrosis factor agents and other new drugs for inflammatory bowel disease. *Bmj* **2017**, *357*, https://doi.org/10.1136/bmj.j2505.
30. Pelechas, E.; Voulgari, P.V.; Drosos, A.A. Preclinical discovery and development of Adalimumab for the treatment of rheumatoid arthritis. *Expert Opinion on Drug Discovery* **2021**, *16*, 227-234, https://doi.org/10.1080/17460441.2021.1846516.
31. Patsalos, O.; Dalton, B.; Leppanen, J.; Ibrahim, M.A.; Himmerich, H. Impact of TNF-α inhibitors on body weight and bmi: A systematic review and meta-analysis. *Frontiers in pharmacology* **2020**, *11*, 481, https://doi.org/10.3389%2Ffphar.2020.00481.
32. De Santis, E.; Melegari, A.; Bonaguri, C.; Sandri, G.; Mascia, M.T.; Gaiani, F.; Pecoraro, V.; De Angelis, G.; Trenti, T. The new test for monitoring infliximab therapy: from laboratory to clinical practice. **2018**, https://pubmed.ncbi.nlm.nih.gov/29431302/.
33. Jawad, M.; Öztürk, K.; Jabir, M.S. TNF-α loaded on gold nanoparticles as promising drug delivery system against proliferation of breast cancer cells. *Materials Today: Proceedings* **2021**, *42*, 3057-3061, https://doi.org/10.1016/j.matpr.2020.12.836.
34. Elliott, M.J.; Maini, R.N.; Feldmann, M.; Kalden, J.R.; Antoni, C.; Smolen, J.; Leeb, B.; Breedveld, F.; Macfarlane, J.; Bijl, J. Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor α (cA2) versus placebo in rheumatoid arthritis. *The Lancet* **1994**, *344*, 1105-1110, https://doi.org/10.1016/s0140-6736(94)90628-9.
35. Mercogliano, M.F.; Bruni, S.; Elizalde, P.V.; Schillaci, R. Tumor necrosis factor α blockade: an opportunity to tackle breast cancer. *Frontiers in oncology* **2020**, *10*, 584, https://doi.org/10.3389%2Ffonc.2020.00584.
36. Wang, Y.; Guo, L.; Yin, X.; McCarthy, E.C.; Cheng, M.I.; Hoang, A.T.; Chen, H.-C.; Patel, A.Y.; Allard Trout, D.; Xu, E. Pathogenic TNF-α drives peripheral nerve inflammation in an Aire-deficient model of autoimmunity. *Proceedings of the National Academy of Sciences* **2022**, *119*, e2114406119, https://doi.org/10.1073/pnas.2114406119.
37. Li, X.; Guo, D.; Chen, Y.; Hu, Y. Toll-like receptors/TNF-α pathway crosstalk and impact on different sites of recurrent myocardial infarction in elderly patients. *BioMed research international* **2022**, *2022*, https://doi.org/10.1155/2022/1280350.
38. Schottelius, A.J.; Moldawer, L.L.; Dinarello, C.A.; Asadullah, K.; Sterry, W.; Edwards III, C.K. Biology of tumor necrosis factor-α–implications for psoriasis. *Experimental dermatology* **2004**, *13*, 193-222, https://doi.org/10.1111/j.0906-6705.2004.00205.x.
39. Daub, H.; Traxler, L.; Ismajli, F.; Groitl, B.; Itzen, A.; Rant, U. The trimer to monomer transition of Tumor Necrosis Factor-Alpha is a dynamic process that is significantly altered by therapeutic antibodies. *Scientific reports* **2020**, *10*, 1-10, https://www.nature.com/articles/s41598-020-66123-5.
40. Dhital, R.; Kavanaugh, A. Autoimmune consequences of biologics. *Journal of Allergy and Clinical Immunology* **2022**, *149*, 48-50, https://doi.org/10.1016/j.jaci.2021.11.011.
41. Danese, S.; Peyrin-Biroulet, L. Evolution of IL-23 Blockade in Inflammatory Bowel Disease. **2022**, *16*, ii1-ii2, https://doi.org/10.1093/ecco-jcc/jjab188.
42. Felis-Giemza, A.; Apanel-Kotarska, A.; Chojnowski, M.; Olesińska, M. Is intra-articular infliximab therapy a good alternative to radionuclide synovectomy for a patient with refractory pigmented villonodular synovitis? *Reumatologia/Rheumatology* **2021**, *59*, 340-345, https://doi.org/10.5114%2Freum.2021.110601.
43. Sharifi, M. The Role of Tumor Necrosis Factor-alpha (TNF-α) in the Pathogenesis of Oral Diseases. *J Dent Oral Health* **2022**, *9*, 1-14, https://www.researchgate.net/profile/Meenakshi-Chellaiah/publication/358530004_JScholar_Publishers_Journal_of_Dentistry_and_Oral_Health_Citation_Marjan_Sharifi_2022_The_Role_of_Tumor_Necrosis_Factor-alpha_TNF-a_in_the_Pathogenesis_of_Oral_Diseases/links/62066adf87866404a1601381/JScholar-Publishers-Journal-of-Dentistry-and-Oral-Health-Citation-Marjan-Sharifi-2022-The-Role-of-Tumor-Necrosis-Factor-alpha-TNF-a-in-the-Pathogenesis-of-Oral-Diseases.pdf.
44. WATANABE, N.; NIITSU, Y.; NEDA, H.; SONE, H.; YAMAUCHI, N.; UMETSU, T.; URUSHIZAKI, I. Antitumor effect of tumor necrosis factor against various primarily cultured human cancer cells. *Japanese Journal of Cancer Research GANN* **1985**, *76*, 1115-1119, https://doi.org/10.20772/cancersci1985.76.11_1115.
45. Dixon, M.L.; De La Vega, A.; Mills, C.; Andrews-Hanna, J.; Spreng, R.N.; Cole, M.W.; Christoff, K. Heterogeneity within the frontoparietal control network and its relationship to the default and dorsal attention networks. *Proceedings of the National Academy of Sciences* **2018**, *115*, E1598-E1607, https://doi.org/10.1073/pnas.1715766115.

46. Wong, G. Elwell JH, Oberley LW, Goeddel DV. Manganous superoxide dismutase is essential for cellular resistance to cytotoxicity of tumor necrosis factor. *Cell* **1989**, *58*, 923-931, https://doi.org/10.1016/0092-8674(89)90944-6.
47. Spriggs, D.R.; Imamura, K.; Rodriguez, C.; Sariban, E.; Kufe, D.W. Tumor necrosis factor expression in human epithelial tumor cell lines. *The Journal of clinical investigation* **1988**, *81*, 455-460, https://doi.org/10.1172%2FJCI113341.
48. Pei, H.; Guo, W.; Peng, Y.; Xiong, H.; Chen, Y. Targeting key proteins involved in transcriptional regulation for cancer therapy: Current strategies and future prospective. *Medicinal Research Reviews* **2022**, https://doi.org/10.1002/med.21886.
49. Gapuzan, M.-E.R.; Schmah, O.; Pollock, A.D.; Hoffmann, A.; Gilmore, T.D. Immortalized fibroblasts from NF-κB RelA knockout mice show phenotypic heterogeneity and maintain increased sensitivity to tumor necrosis factor α after transformation by v-Ras. *Oncogene* **2005**, *24*, 6574-6583, https://www.nature.com/articles/1208809.
50. Wang, C.-Y.; Cusack, J.C.; Liu, R.; Baldwin, A.S. Control of inducible chemoresistance: enhanced anti-tumor therapy through increased apoptosis by inhibition of NF-κB. *Nature medicine* **1999**, *5*, 412-417, https://doi.org/10.1038/7410.
51. Zhu, J.; Xin, Y.; Liu, X.; Wang, Y.; Liu, Y. Nimotuzumab enhances the sensitivity of non-small cell lung cancer cells to tumor necrosis factor-α by inhibiting the nuclear factor-κB signaling pathway. *Experimental and Therapeutic Medicine* **2018**, *15*, 3345-3351, https://doi.org/10.3892/etm.2018.5856.
52. Burow, M.E.; Weldon, C.B.; Tang, Y.; Navar, G.L.; Krajewski, S.; Reed, J.C.; Hammond, T.G.; Clejan, S.; Beckman, B.S. Differences in susceptibility to tumor necrosis factor α-induced apoptosis among MCF-7 breast cancer cell variants. *Cancer research* **1998**, *58*, 4940-4946, https://pubmed.ncbi.nlm.nih.gov/9810003/.
53. Pagliacci, M.C.; Fumi, G.; Migliorati, G.; Grignani, F.; Riccardi, C.; Nicoletti, I. Cytostatic and cytotoxic effects of tumor necrosis factor alpha on MCF-7 human breast tumor cells are differently inhibited by glucocorticoid hormones. *Lymphokine and cytokine research* **1993**, *12*, 439-447, https://pubmed.ncbi.nlm.nih.gov/8123760/.
54. Müller-Decker, K.; Leder, C.; Neumann, M.; Neufang, G.; Marks, F.; Fürstenberger, G.; Bayerl, C.; Schweizer, J. Expression of cyclooxygenase isozymes during morphogenesis and cycling of pelage hair follicles in mouse skin: precocious onset of the first catagen phase and alopecia upon cyclooxygenase-2 overexpression. *Journal of investigative dermatology* **2003**, *121*, 661-668, https://doi.org/10.1046/j.1523-1747.2003.12473.x.
55. Ememe, M.; Abdullahi, U.; Sackey, A. Modulatory Effects of Resveratrol Supplementation on Inflammatory Markers in Ageing and Lame Horses. *Int. J. Anim. Veter. Adv* **2015**, *7*, 67-72, http://dx.doi.org/10.19026/ijava.7.1693.
56. Whiteside, T.L. Cytokine assays. *Biotechniques* **2002**, *33*, S4-S15, https://www.future-science.com/doi/pdf/10.2144/Oct0202.
57. Fries, W.; Muja, C.; Crisafulli, C.; Costantino, G.; Longo, G.; Cuzzocrea, S.; Mazzon, E. Infliximab and Etanercept are equally effective in reducing enterocyte APOPTOSIS in experimental colitis. *International Journal of Medical Sciences* **2008**, *5*, 169, https://doi.org/10.7150/ijms.5.169.
58. Mitoma, H.; Horiuchi, T.; Tsukamoto, H. Binding activities of Infliximab and Etanercept to transmembrane tumor necrosis factor-α. *Gastroenterology* **2004**, *126*, 934-935, https://doi.org/10.1053/j.gastro.2004.01.036.
59. Ackermann, C.; Kavanaugh, A. Tumor necrosis factor as a therapeutic target of rheumatologic disease. *Expert opinion on therapeutic targets* **2007**, *11*, 1369-1384, https://doi.org/10.1517/14728222.11.11.1369.
60. Guo, Y.; Hu, K.; Li, Y.; Lu, C.; Ling, K.; Cai, C.; Wang, W.; Ye, D. Targeting TNF-α for COVID-19: Recent Advanced and Controversies. *Frontiers in Public Health* **2022**, *10*, 833967, https://www.frontiersin.org/articles/10.3389/fpubh.2022.833967/pdf.
61. Dixon, W.; Hyrich, K.; Watson, K.; Lunt, M.; Galloway, J.; Ustianowski, A.; Symmons, D.; Consortium, B.C.C. Drug-specific risk of tuberculosis in patients with rheumatoid arthritis treated with anti-TNF therapy: results from the British Society for Rheumatology Biologics Register (BSRBR). *Annals of the rheumatic diseases* **2010**, *69*, 522-528, https://pubmed.ncbi.nlm.nih.gov/19854715/.
62. Sandborn, W.J.; Hanauer, S.B.; Katz, S.; Safdi, M.; Wolf, D.G.; Baerg, R.D.; Tremaine, W.J.; Johnson, T.; Diehl, N.N.; Zinsmeister, A.R. Etanercept for active Crohn's disease: a randomized, double-blind, placebo-controlled trial. *Gastroenterology* **2001**, *121*, 1088-1094, https://pubmed.ncbi.nlm.nih.gov/11677200/.
63. Salmon-Céron, D.; Tubach, F.; Lortholary, O.; Chosidow, O.; Bretagne, S.; Nicolas, N.; Cuillerier, E.; Fautrel, B.; Michelet, C.; Morel, J. Drug-specific risk of non-tuberculosis opportunistic infections in patients receiving anti-TNF therapy reported to the 3-year prospective French RATIO registry. *Annals of the rheumatic diseases* **2011**, *70*, 616-623, https://ard.bmj.com/content/70/4/616.
64. Byng-Maddick, R.; Turner, C.T.; Pollara, G.; Ellis, M.; Guppy, N.J.; Bell, L.C.; Ehrenstein, M.R.; Noursadeghi, M. Tumor necrosis factor (TNF) bioactivity at the site of an acute cell-mediated immune response is preserved in rheumatoid arthritis patients responding to anti-TNF therapy. *Frontiers in Immunology* **2017**, *8*, 932, https://pubmed.ncbi.nlm.nih.gov/28824652/.

65. Nadkarni, S.; Mauri, C.; Ehrenstein, M.R. Anti–TNF-α therapy induces a distinct regulatory T cell population in patients with rheumatoid arthritis via TGF-β. *The Journal of experimental medicine* **2007**, *204*, 33-39, https://pubmed.ncbi.nlm.nih.gov/17200409/.
66. Harris, J.; Keane, J. How tumour necrosis factor blockers interfere with tuberculosis immunity. *Clinical & Experimental Immunology* **2010**, *161*, 1-9, https://pubmed.ncbi.nlm.nih.gov/20491796/
67. Belmellat, N.; Semerano, L.; Segueni, N.; Damotte, D.; Decker, P.; Ryffel, B.; Quesniaux, V.; Boissier, M.-C.; Assier, E. Tumor necrosis factor-alpha targeting can protect against arthritis with low sensitization to infection. *Frontiers in immunology* **2017**, *8*, 1533, https://www.frontiersin.org/articles/10.3389/fimmu.2017.01533/full.
68. Neubauer, S.; Cifaldi, M.; Mittendorf, T.; Ganguli, A.; Wolff, M.; Zeidler, J. Biologic TNF inhibiting agents for treatment of rheumatoid arthritis: persistence and dosing patterns in Germany. *Health Economics Review* **2014**, *4*, 1-11, https://pubmed.ncbi.nlm.nih.gov/26208932/.
69. Desai, R.J.; Thaler, K.J.; Mahlknecht, P.; Gartlehner, G.; McDonagh, M.S.; Mesgarpour, B.; Mazinanian, A.; Glechner, A.; Gopalakrishnan, C.; Hansen, R.A. Comparative risk of harm associated with the use of targeted immunomodulators: a systematic review. *Arthritis Care & Research* **2016**, *68*, 1078-1088, https://pubmed.ncbi.nlm.nih.gov/26663412/.
70. Flouri, I.; Markatseli, T.E.; Voulgari, P.V.; Boki, K.A.; Papadopoulos, I.; Settas, L.; Zisopoulos, D.; Skopouli, F.N.; Iliopoulos, A.; Bertsias, G.K. Comparative effectiveness and survival of Infliximab, Adalimumab, and Etanercept for rheumatoid arthritis patients in the Hellenic Registry of Biologics: Low rates of remission and 5-year drug survival. In Proceedings of the Seminars in arthritis and rheumatism, 2014; pp. 447-457, https://read.qxmd.com/read/24012040/comparative-effectiveness-and-survival-of-infliximab-adalimumab-and-etanercept-for-rheumatoid-arthritis-patients-in-the-hellenic-registry-of-biologics-low-rates-of-remission-and-5-year-drug-survival.
71. Leon, L.; Rodriguez-Rodriguez, L.; Rosales, Z.; Gomez, A.; Lamas, J.; Pato, E.; Jover, J.; Abasolo, L. Long-term drug survival of biological agents in patients with rheumatoid arthritis in clinical practice. *Scandinavian journal of rheumatology* **2016**, *45*, 456-460, https://pubmed.ncbi.nlm.nih.gov/27115843/.
72. Wolbink, G.J.; Vis, M.; Lems, W.; Voskuyl, A.E.; De Groot, E.; Nurmohamed, M.T.; Stapel, S.; Tak, P.P.; Aarden, L.; Dijkmans, B. Development of antiinfliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis. *Arthritis & Rheumatism* **2006**, *54*, 711-715, https://www.langanbach.ie/wp-content/uploads/2018/03/Antiinfliximab_Wolbink-et-al_arthr_Rheum_2006.pdf.
73. Matucci, A.; Cammelli, D.; Cantini, F.; Goletti, D.; Marino, V.; Milano, G.M.; Scarpa, R.; Tocci, G.; Maggi, E.; Vultaggio, A. Influence of anti-TNF immunogenicity on safety in rheumatic disease: a narrative review. *Expert opinion on drug safety* **2016**, *15*, 3-10, https://pubmed.ncbi.nlm.nih.gov/27924646/.
74. Matucci, A.; Pratesi, S.; Petroni, G.; Nencini, F.; Virgili, G.; Milla, M.; Maggi, E.; Vultaggio, A. Allergological *in vitro* and *in vivo* evaluation of patients with hypersensitivity reactions to Infliximab. *Clinical & Experimental Allergy* **2013**, *43*, 659-664, https://pubmed.ncbi.nlm.nih.gov/23711128/.
75. Favalli, E.G.; Pregnolato, F.; Biggioggero, M.; Becciolini, A.; Penatti, A.E.; Marchesoni, A.; Meroni, P.L. Twelve-year retention rate of first-line tumor necrosis factor inhibitors in rheumatoid arthritis: real-life data from a local registry. *Arthritis care & research* **2016**, *68*, 432-439, https://pubmed.ncbi.nlm.nih.gov/26556048/.
76. Souto, A.; Maneiro, J.R.; Gomez-Reino, J.J. Rate of discontinuation and drug survival of biologic therapies in rheumatoid arthritis: a systematic review and meta-analysis of drug registries and health care databases. *Rheumatology* **2016**, *55*, 523-534, https://pubmed.ncbi.nlm.nih.gov/26490106/.
77. Papadopoulos, C.G.; Gartzonikas, I.K.; Pappa, T.K.; Markatseli, T.E.; Migkos, M.P.; Voulgari, P.V.; Drosos, A.A. Eight-year survival study of first-line tumour necrosis factor α inhibitors in rheumatoid arthritis: real-world data from a university centre registry. *Rheumatology advances in practice* **2019**, *3*, rkz007, https://pubmed.ncbi.nlm.nih.gov/31431995/.
78. Mitoma, H.; Horiuchi, T.; Tsukamoto, H.; Tamimoto, Y.; Kimoto, Y.; Uchino, A.; To, K.; Harashima, S.I.; Hatta, N.; Harada, M. Mechanisms for cytotoxic effects of anti–tumor necrosis factor agents on transmembrane tumor necrosis factor α–expressing cells: comparison among Infliximab, Etanercept, and Adalimumab. *Arthritis & Rheumatism* **2008**, *58*, 1248-1257, https://pubmed.ncbi.nlm.nih.gov/18438840/.
79. Nesbitt, A.; Fossati, G.; Bergin, M.; Stephens, P.; Stephens, S.; Foulkes, R.; Brown, D.; Robinson, M.; Bourne, T. Mechanism of action of certolizumab pegol (CDP870): *in vitro* comparison with other anti-tumor necrosis factor α agents. *Inflammatory bowel diseases* **2007**, *13*, 1323-1332, https://academic.oup.com/ibdjournal/article/13/11/1323/4652888.
80. Zou, J.; Rudwaleit, M.; Brandt, J.; Thiel, A.; Braun, J.; Sieper, J. Down-regulation of the nonspecific and antigen-specific T cell cytokine response in ankylosing spondylitis during treatment with Infliximab. *Arthritis & Rheumatism* **2003**, *48*, 780-790, https://pubmed.ncbi.nlm.nih.gov/12632433/.
81. Dong, L.; Jiang, H.; Chen, K.; Xie, J.; An, Q.; Deng, F.; Sun, Z.; Liu, Y.; Zhou, J.; Zhang, L. Mannan-Binding Lectin Deficiency Limits Inflammation-induced Myeloid-Derived Suppressor Cells Expansion via Modulating Tumor Necrosis Factor Alpha-triggered Apoptosis. *International journal of biological sciences* **2022**, *18*, 1580, https://pubmed.ncbi.nlm.nih.gov/35280697/.

82. Zhang, R.; Xue, T.; Shao, A.; Lang, Y.; Qin, C.; Zhao, M.; Kuang, Y.; Yu, Z.; Geng, Y.; Zhao, C. Bclaf1 regulates c-FLIP expression and protects cells from TNF-induced apoptosis and tissue injury. *EMBO reports* **2022**, *23*, e52702, https://pubmed.ncbi.nlm.nih.gov/34693625/.
83. Tobin, D.; Van Hogerlinden, M.; Toftgård, R. UVB-induced association of tumor necrosis factor (TNF) receptor 1/TNF receptor-associated factor-2 mediates activation of Rel proteins. *Proceedings of the National Academy of Sciences* **1998**, *95*, 565-569, https://pubmed.ncbi.nlm.nih.gov/9435232/.
84. Grell, M.; Douni, E.; Wajant, H.; Löhden, M.; Clauss, M.; Maxeiner, B.; Georgopoulos, S.; Lesslauer, W.; Kollias, G.; Pfizenmaier, K. The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDa tumor necrosis factor receptor. *Cell* **1995**, *83*, 793-802, https://pubmed.ncbi.nlm.nih.gov/8521496/.
85. Kokolakis, G.; Sabat, R.; Krüger-Krasagakis, S.; Eberle, J. Ambivalent effects of tumor necrosis factor alpha on apoptosis of malignant and normal human keratinocytes. *Skin Pharmacology and Physiology* **2021**, *34*, 94-102, https://www.karger.com/Article/FullText/513725.